Expression and Functional Importance of Discoidin Domain Receptor 1 in Dentoalveolar Tissues

THESIS

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By

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Abstract

Collagen fibrils are abundant in all parts of the body and function to strengthen and support the extracellular framework in animal tissue. Collagen is a main component of fascia, cartilage, ligaments, tendons, bones and teeth. Like other connective tissues in the body, dentoalveolar tissues, including gingiva, dentin, cementum, periodontal ligament (PDL), and alveolar bone, are composed primarily of type I collagen as the major protein component. Discoidin domain receptors (DDR1 and DDR2) are receptors for several collagen types. They have been found to regulate collagen fibrillization and deposition in extracellular matrix (ECM) remodeling via tyrosine kinase activity, thereby modulating cell proliferation and affecting development of tissues. There have been limited studies on the expression and function of DDR1 during dentoalveolar development. In this study, we defined the expression patterns of DDR1 in dentoalveolar tissues and investigated the functional importance of DDR1 in tooth development using a DDR1-deficient mouse model. Expression of DDR1 was identified in the epithelial tissues including the enamel organ and gingiva, but expression was not detected in any ectomesenchymal cells of the pulp, dentin, or periodontium. Conversely, DDR2 expression was identified in odontoblasts and osteoblasts. Radiography revealed no major differences in *Ddr1*$^{-/-}$ vs. WT dentoalveolar structures; however, *Ddr1*$^{-/-}$ mandibles featured defective condyles and

abnormal subchondral bone structure. Histomorphometry measurements of oral, dental, and periodontal tissues did not identify significant anatomic differences, including gingival epithelium. These studies indicate distinct expression patterns for DDR1 and DDR2, suggesting dissimilar functions in dentoalveolar tissues. Our findings indicate signs of osteoarthritis in the DDR1-deficient condylar process and periodontitis in the 2nd and 3rd molars of the mandible. This study suggests the importance of DDR1 in arthritis and periodontal function and may potentially inform disease mechanisms, wound healing, novel dental and periodontal therapies, and reconstruction of bone for dental and orthopedic purposes.

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Chapter 1: Introduction

Background

The majority of collagen in the body consists of types I, II, and III, however, type I collagen is one of the most predominant proteins that is found in mammalian connective tissues. Though all the types of collagen assemble into a triple-helical structure, the ability to form and organize the fibers differs from each type. For example, type I collagen predominates in bone, type II is the major collagen in cartilage, and type IV forms the basic network of all basal laminae (Lodish et al, 2000). During remodeling of the extracellular matrix (ECM), the monomeric triple-helical collagen molecules secreted by the cells will rapidly assemble into microfibrils which then combine to form collagen fibrils and fibers. Several factors in the ECM, including non-collagenous proteins (NCPs), can modulate collagen fibrillogenesis. The NCPs, discoidin domain receptors (DDR1 and DDR2), are receptor tyrosine kinases that are activated upon binding to collagen (Srivastava et al, 1997). The major collagen-binding domain of DDRs has been deduced to be their extracellular domain (ECD) (Yeung et al, 2013; Mihai et al, 2009). Upon collagen binding, DDRs undergo a tyrosine auto-phosphorylation that initiates distinct signaling pathways in order to regulate a variety of cell-collagen interactions. Both DDR1 and DDR2 control cell migration and adhesion and play an important role in

physiological processes like wound healing (DDR2) and immune responses (DDR1). Various studies with DDR-deficient mice also show these receptors may play a key role in inflammatory and fibrotic responses (Fu et al., 2013).

Collagen forms connective fibers in tissues such as skin, ligaments, cartilage, bones, and teeth. In bones and teeth, which are composites of organic (ECM) and inorganic (hydroxyapatite) phases, collagen fibers contribute to the mechanical strength and toughness necessary for these hard tissues to properly function (McKee et al, 2013). The organic ECM of bones and teeth is composed of 90% type I collagen, with lesser amounts of other types. The dentoalveolar complex (i.e. teeth and surrounding connective tissues) is composed of four distinct mineralized tissues: enamel, dentin, cementum, and bone. The periodontal tissues that attach the tooth root include mineralized tissues, cementum and alveolar bone, and the non-mineralized periodontal ligament (PDL), which is rich in fibroblasts that direct its ECM metabolism (Beertsen et al, 1997). The PDL is in a constant state of remodeling as reflected by radio autographic studies that have indicated a very high collagen turnover rate The alveolar bone that surrounds the PDL is the most rapidly remodeling bone in the body and the collagen fibers located within this bone are angled so as to respond to mechanical loads transmitted from various directions (Sodek and McKee, 2000). Diseases resulting from mutations in collagen or collagen-related molecules (e.g. osteogenesis imperfecta) or ECM remodeling (e.g. Winchester syndrome) are known to cause defects in dentoalveolar development and function (Foster et al, 2014;

Xu et al, 2016). Currently, the expression and importance of DDR1 is unknown during dentoalveolar development and function.

Research Significance

Periodontal tissues are essential for tooth function, however, these can be lost to disease and trauma. Genetic polymorphisms can affect genes that are involved in periodontal tissue remodeling, degradation, and inflammation, thereby conferring susceptibility to periodontal disease (Baldi et al, 2009). Therapies to regenerate periodontal tissues are currently unpredictable, in part due to lack of insights into formation and remodeling of these tissues (Bosshardt and Sculean, 2009). These studies will begin to elucidate the roles of DDR1 in tooth formation and periodontal ECM remodeling, providing new information that may inform future novel therapies aimed at improving oral health and prevention of oral degenerative diseases.

Overview of Thesis

Based on the highly collagenous nature of dentoalveolar tissues and high rates of remodeling of PDL and alveolar bone, we hypothesize that DDR1 is expressed in the respective cells at key stages of development and is involved in directing formation and remodeling of the periodontal tissues. For my thesis, I will use a variety of approaches to determine if genetic deletion of DDR1 alters development and function of dental and periodontal tissues. My specific research goals include:

Aim 1: To define spatiotemporal expression of DDR1 during tooth formation.

Aim 2: To analyze development and function of dentoalveolar tissues in DDR1 knock-out (*Ddr1*$^{-/-}$) mice.

Chapter 2: Methodology

Aim 1: To define spatiotemporal expression of DDR1 during tooth formation

Mice: All animal studies were approved by Institutional Animal Care and Use Committee (IACUC; The Ohio State University, Columbus, OH). Normal mice (strain: C57BL6/J from Jackson Laboratories, Bar Harbor, ME) at several ages during odontogenesis and after completion of tooth formation (8 to 60 days postnatal; dpn) were used to assay *Ddr1* mRNA and DDR1 protein expression in dentoalveolar tissues. Mice were euthanized by carbon dioxide and mandibles were collected for the described studies.

Histology: Dissected mouse mandibles were fixed in Bouin's solution for 24 hours, decalcified in acetic acid/formalin/sodium chloride (AFS) solution at 4°C for 3-4 weeks, and embedded in paraffin for microtome sectioning into 5 μm thick coronal and sagittal slices (Foster, 2012).

In situ hybridization (ISH): ISH was used on histological sections to localize *Ddr1* and *Ddr2* mRNA expression during tooth development. The RNA probes for *Ddr1* and *Ddr2* were purchased from ACD RNAscope (Newark, CA, USA). ISH was performed under

the manufacturer protocol on pretreated sections to allow for access to target RNA. The probes were hybridized to the target mRNA, the signal was amplified, and Fast Red substrate was added to detect the target mRNA as a red reaction color.

Aim 2: To analyze development and function of dentoalveolar tissues in *Ddr1*$^{-/-}$ mice.

Mice: All animal studies were approved by IACUC (The Ohio State University, Columbus, OH). Mice where DDR1 is genetically ablated (*Ddr1*$^{-/-}$) are currently in use in the Agarwal lab and were used to generate tissues for analysis under Aim 2. Wild type (WT) controls included age- and sex-matched littermates. Tissues from WT and *Ddr1*$^{-/-}$ were harvested for analysis at 4-6 months old.

Radiography and microcomputed tomography (micro-CT): Fixed and undecalcified crania and mandibles were scanned with an MX-20 digital x-ray system (Faxitron X-ray Corp., Chicago, IL, USA) (Foster et al, 2015). Qualitative and quantitative micro-CT approaches were used to analyze mineralized tissue differences in *Ddr1*$^{-/-}$ vs. WT (Foster et al, 2015). Samples were scanned in a µCT 50 (Scanco Medical, Bassersdorf, Switzerland) at 70 kVp, 76 µA, 0.5 Al Filter, 900 ms integration time, and 6 µm voxel size. DICOM images were uploaded to AnalyzePro 1.0 (AnalyzeDirect, Overland Park, KS) and calibrated to mg/cm^3 HA from 5 known densities of mg/cm^3. Mandibles were oriented to a standard orientation using the first molar for anatomical landmarks. The region of interest (ROI) was then determined using the standard orientation. Enamel was

segmented at 1600 mg/cm^3, and dentin and bone at 650 mg/cm^3. Density and volume were analyzed for all parameters. For qualitative observation, the subchondral and underlying condylar bone regions were distinguished and highlighted via differences detected by the computer under selected settings (Figure 1). For quantitative analysis, the ROIs were segmented layer-by-layer to distinguish the components of the tooth. Measurements for n=6 mice per genotype were analyzed by two-tailed t-test ($\alpha = 0.05$) to determine statistical significance using GraphPad Prism software (GraphPad Prism version 6.00, GraphPad Software, La Jolla, CA, USA, www.graphpad.com).

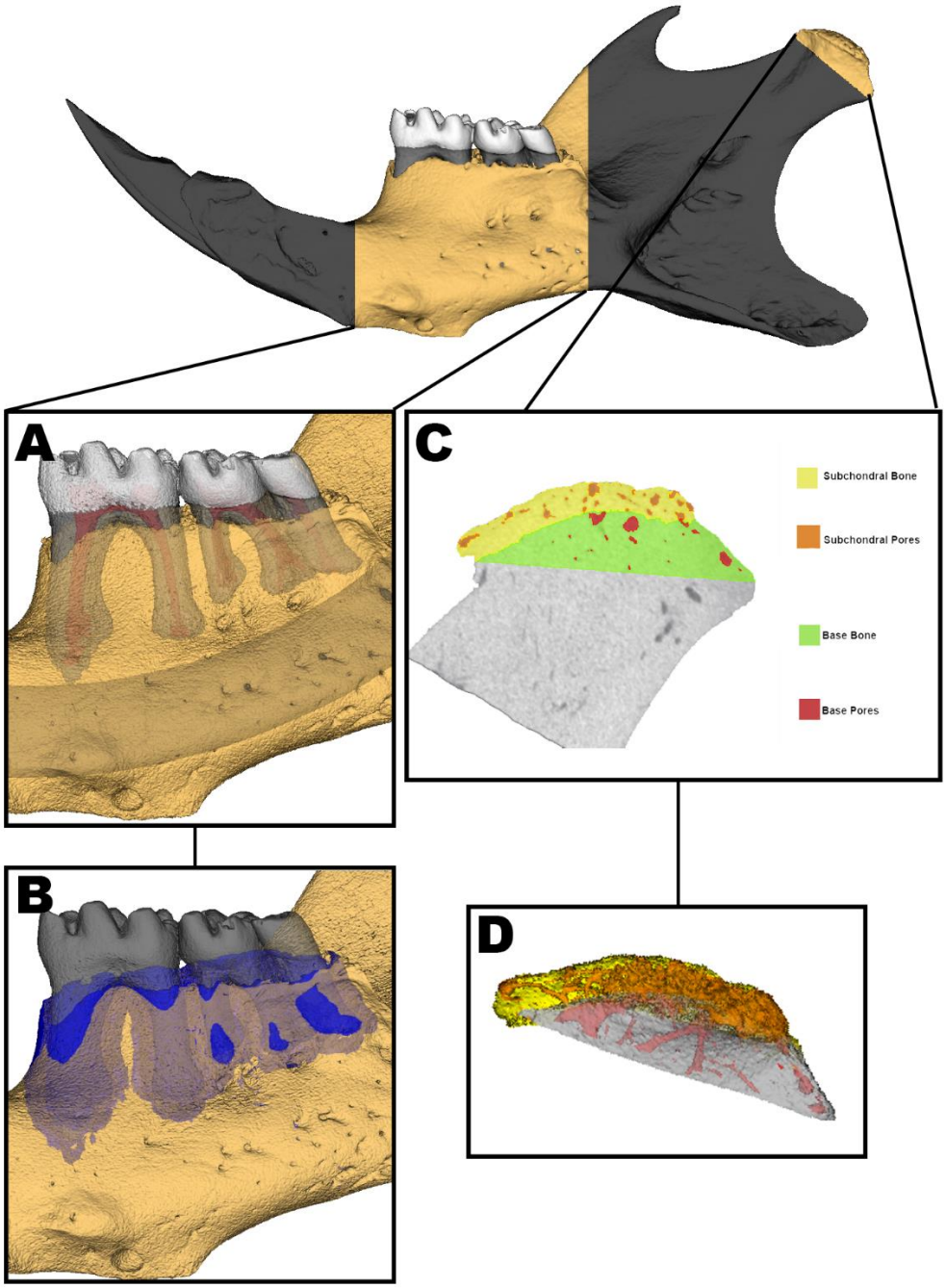

Figure 1. Method of analysis via micro-computed tomography. Regions of interest in the condyle and dentoalveolar tissues are described. (A) Area of analysis between 1st, 2nd, 3rd molars in 3D-generated render. (B) 3D render displaying supporting bone (blue). Boundaries were set 250 µm out from the dentin to represent the bone-tooth attachment. (C) Regions of the condyle analyzed (D) 3D representation of the condyle regions.

Histology: Fixation, decalcification, and sectioning of tissues were performed as described under Aim 1. Hematoxylin and eosin (H&E) staining was used to observe tissue morphology and ImageJ was used to perform histomorphometric measurements comparing WT and *Ddr1*$^{-/-}$ samples (n = 15) (Foster, 2012). Measurements were analyzed by two-tailed t-test ($\alpha = 0.05$) to determine statistical significance using GraphPad Prism software. Picrosirius red staining was performed to analyze collagen fiber organization in the PDL (Lattouf et al, 2014). Picrosirius red stained sections were observed under polarized light microscopy.

Immunohistochemistry (IHC): IHC was performed to analyze markers of affected tissues. IHC was performed as previously described (Foster, 2012; Foster et al, 2015). Briefly, IHC was performed on paraffin sections using an avidin–biotinylated peroxidase enzyme complex-based kit (Vectastain Elite; Vector Labs, Burlingame, CA, USA) with a 3-amino-9-ethylcarbazole substrate (Vector Labs) to produce a red reaction product indicating antigen localization. Counterstaining was performed by dipping slides in hematoxylin for 20 sec and then placing under gently running water for 5-10 min, followed by cover slipping with aqueous mounting solution. The primary antibody used was rat IgG monoclonal to mouse neutrophils (NIMP; Abcam, Cambridge, MA, USA).

Chapter 3: Results

Ddr1 is expressed in epithelial tissues during dentoalveolar development

The spatiotemporal expression of *Ddr1* was mapped during dentoalveolar development using ISH that employed RNA probes. Sagittal sections at 8 dpn revealed *Ddr1* expression specifically in the enamel organs of first and second molars, and in the epithelial cap of the third molar tooth bud (Figure 2A, B, E, F). *Ddr1* was also localized to the basal cell layer of the oral epithelium layer at this age (Figure 2B). These tissues are both epithelial in origin, indicating that *Ddr1* expression is specifically associated with ectoderm-derived cells during developmental stages of the tooth. No *Ddr1* expression was noted in the ectomesenchymal dental tissues, including pulp, dentin, dental follicle, or surrounding alveolar bone. At the later age of 14 dpn, *Ddr1* was also seen consistently in the enamel organ and basal cell layer (Figure 2C, D). At 26 dpn, after the first molar tooth had erupted, *Ddr1* mRNA was noted in the basal layer of the junctional epithelium, where gingiva attaches to the tooth surface (Figure 2G, H). Expression patterns at 60 dpn matched those described for 26 dpn (data not shown).

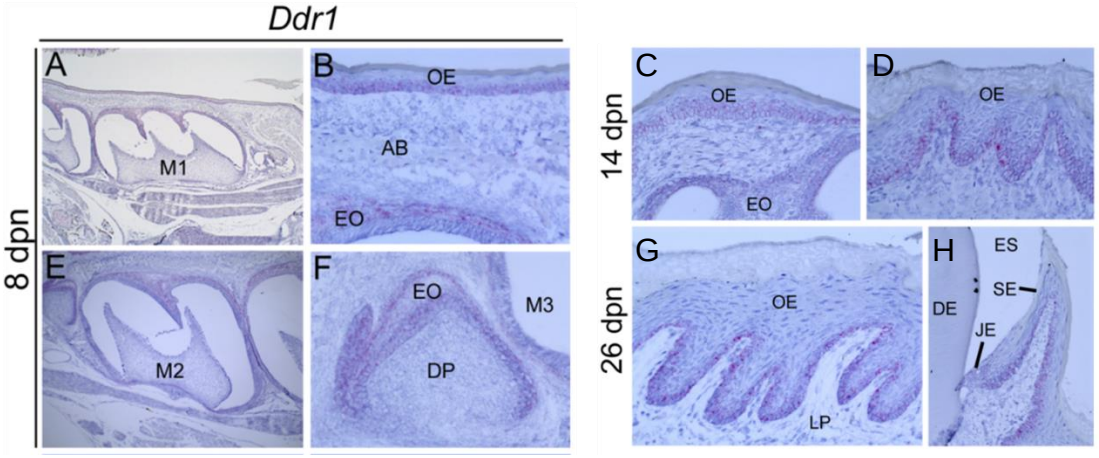

Figure 2. *In situ* hybridization depicting spatial expression of Ddr1 mRNA. RNA probes used to localize Ddr1 mRNA indicate epithelial expression at (A, B, E, F) 8 dpn, (C, D) 14 dpn, and (G, H) 26 dpn. M1=First molar; M2=Second molar; M3=Third molar; OE=Oral epithelium; AB=Alveolar bone; EO=Enamel organ; DP=Dental papilla; LP=Lamina propria; ES=Enamel space; SE=Sulcular epithelium; JE=Junctional epithelium.

Ddr2 expression was also determined by ISH. In contrast to the strictly epithelial expression of *Ddr1*, *Ddr2* mRNA was observed in ectomesenchymal tissues at all ages assayed (data not shown).

Osteoarthritis in the *Ddr1*$^{-/-}$ mouse condyle

Radiographs revealed a grossly normal mouse mandible in 6-month old *Ddr1*$^{-/-}$ mice compared to WT controls (Figure 3). Some radiolucent regions consistent with bone loss were noted around molars of *Ddr1*$^{-/-}$ mice vs. WT controls. The most prominent phenotype observed was an enlarged and irregular condylar process in both female and male *Ddr1*$^{-/-}$ mice compared to WT controls.

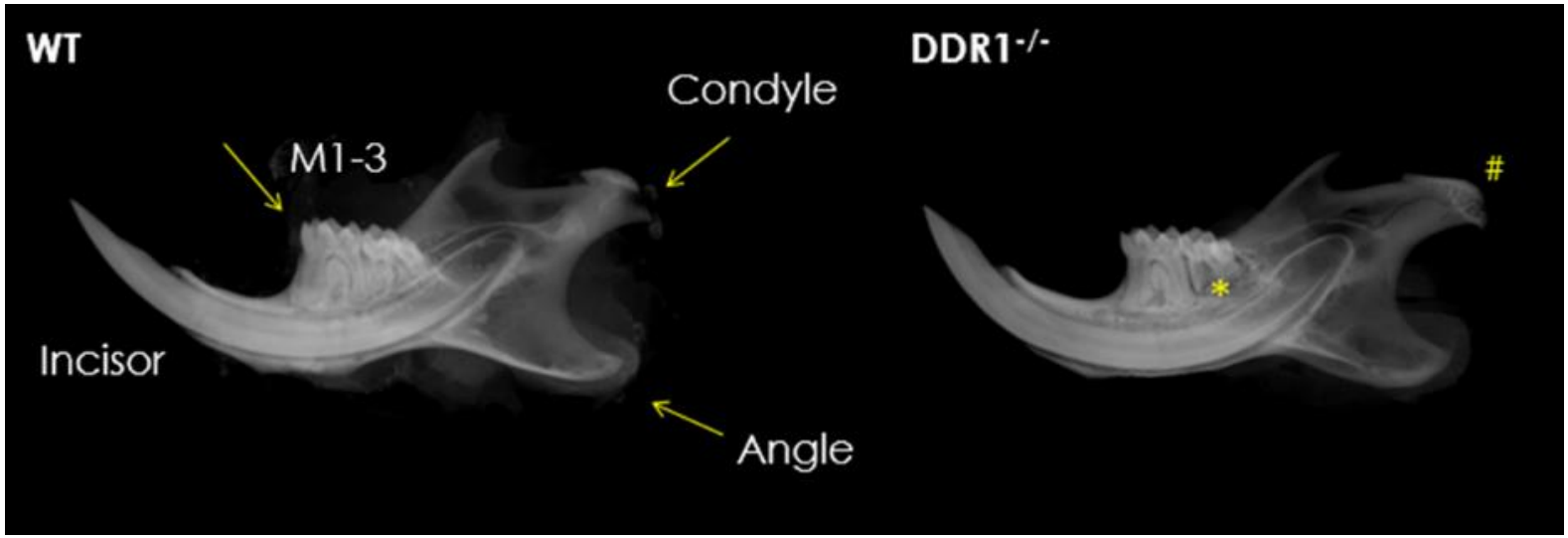

Figure 3. Radiographs depicting 6-month-old mouse mandibles. Compared to WT controls, *Ddr1*$^{-/-}$ mouse mandibles exhibit large and irregular condylar processes (yellow #) and evidence of periodontal bone loss (*), both of which were observed in both males and females.

Micro-CT was performed to further analyze the condylar process in *Ddr1*$^{-/-}$ mice. A 3D render of WT and *Ddr1*$^{-/-}$ condyles created from micro-CT data revealed that *Ddr1*$^{-/-}$ mice had an enlarged condyle with larger subchondral and underlying condylar bone areas as well as an increased basal bone pore area suggesting more vascularization to the region (Figure 4). The subchondral bone boundaries were set 250 μm from the condylar surface while the base bone of the ramus beneath that region was analyzed separately. The subchondral bone is the layer of bone that is just below the cartilage of the temporomandibular joint (TMJ) and is critical for acting as a shock absorber that interfaces with the glenoid fossa of the cranium to define the temporomandibular joint (TMJ). The analysis was conducted to measure bone volume (BV), total volume (TV), and bone volume to total volume ratio (BV/TV) for both highlighted regions. In the subchondral bone of *Ddr1*$^{-/-}$ vs. WT condyles, we found a statistically significant more than 60% increase in BV ($p < 0.01$) and almost 100% increased TV ($p < 0.01$), suggesting increased bone deposition. However, BV/TV was found to be significantly decreased in *Ddr1*$^{-/-}$ subchondral bone by almost 15% ($p < 0.001$). The BV/TV ratio indicates how much of the bone is represented in the total bone mass and because this is significantly lower, this indicates that there are likely more porous areas within the structure, possibly from increased vascularization. The base bone of *Ddr1*$^{-/-}$ vs. WT condyles showed the same trends, with almost 100% significantly increased BV ($p < 0.01$), more than 50% increased TV ($p < 0.01$), and about 10% decreased BV/TV ($p <$

0.01). This combination of enlarged and dystrophic condyle and increased space for vascularization may indicate that the region is inflamed, causing more immune cells to invade the area.

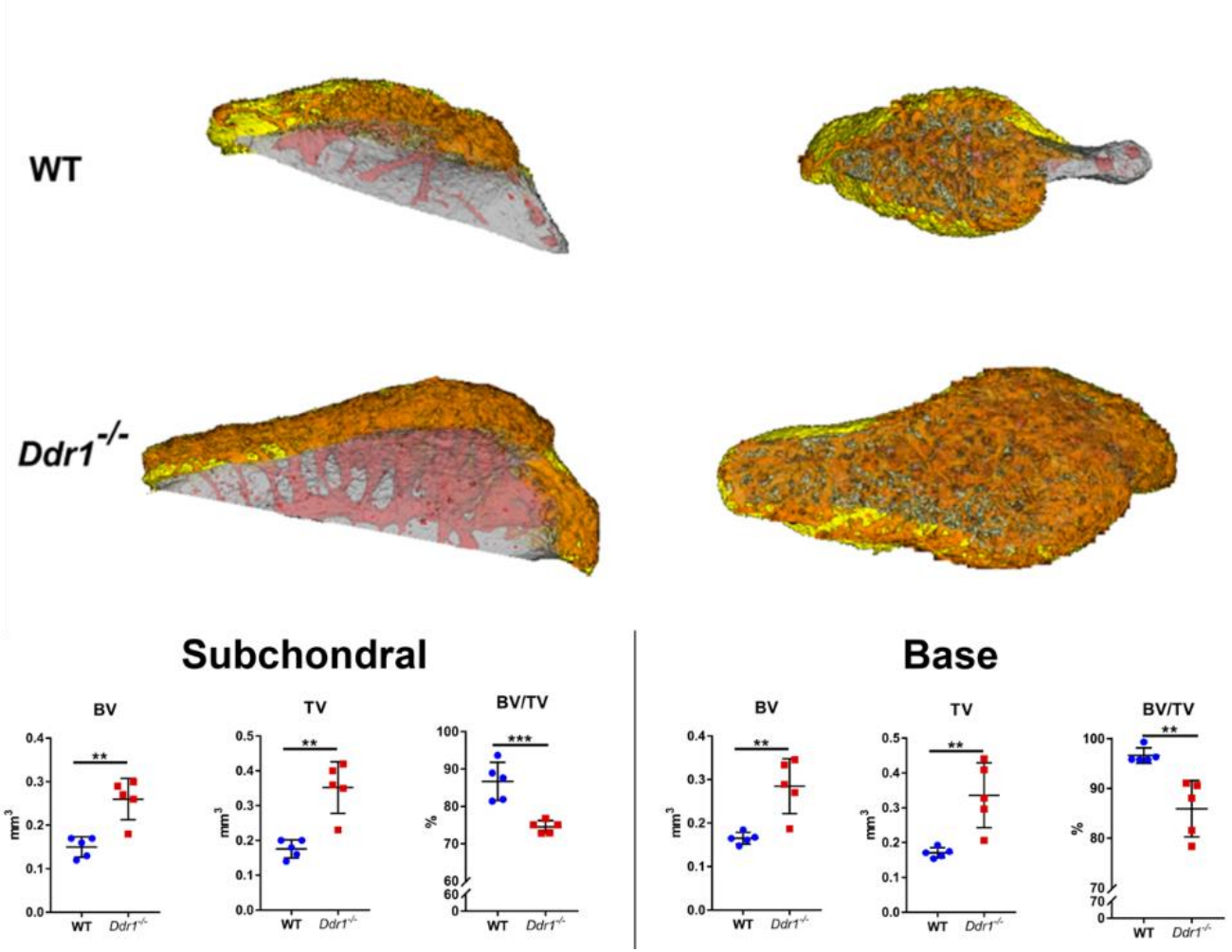

Figure 4. 3D render of condyles and condylar bone analysis. Analysis of n=5 mouse condyles at 4-6 months of age shows significantly increased bone volume (BV), total volume (TV), and decreased BV/TV ratio in both subchondral and base bone in the condylar processes of *Ddr1*$^{-/-}$ vs. WT mice.

Ddr1$^{-/-}$ mice exhibit periodontal inflammation and alveolar bone loss

Radiolucent regions noted by radiography were further explored by micro-CT and histological analysis. The 3D render of the molar region of WT and *Ddr1*$^{-/-}$ mandibles revealed loss of surrounding alveolar bone between the 1st and 3rd molars to form a deep trench (Figure 5A). The supporting bone around the molars, shown in sagittal, coronal, and transverse section orientations, is decreased in the *Ddr1*$^{-/-}$ mice vs. WT controls, and mainly affected the mesial root of the 2nd molar and extending up until the distal portion of the 3rd molar (Figure 5B). The supporting bone in this affected region in *Ddr1*$^{-/-}$ mice sometimes disappeared nearly completely (as in the representative image in Figure 5) and the adjacent alveolar bone region was typically porous and receded compared to WT. The bone volume and densities were quantified to show a statistically significant overall alveolar bone loss of 14% for alveolar bone ($p < 0.0001$) and nearly 25% loss for supporting bone ($p < 0.0001$) in *Ddr1*$^{-/-}$ mice vs. WT controls (Figure 5C). In one *Ddr1*$^{-/-}$ mouse, periodontal bone loss was so extensive as to result in the loss of the 3rd molar (green arrow) (Figure 5D). Histology revealed massive infiltration of neutrophils (red arrow) into the periodontal regions of *Ddr1*$^{-/-}$ mandibles (Figure 5E), confirmed by anti-neutrophil IHC (data not shown). This large influx of inflammatory cells indicates substantial inflammation and immune response in this region where bone loss was also observed by radiology and histology.

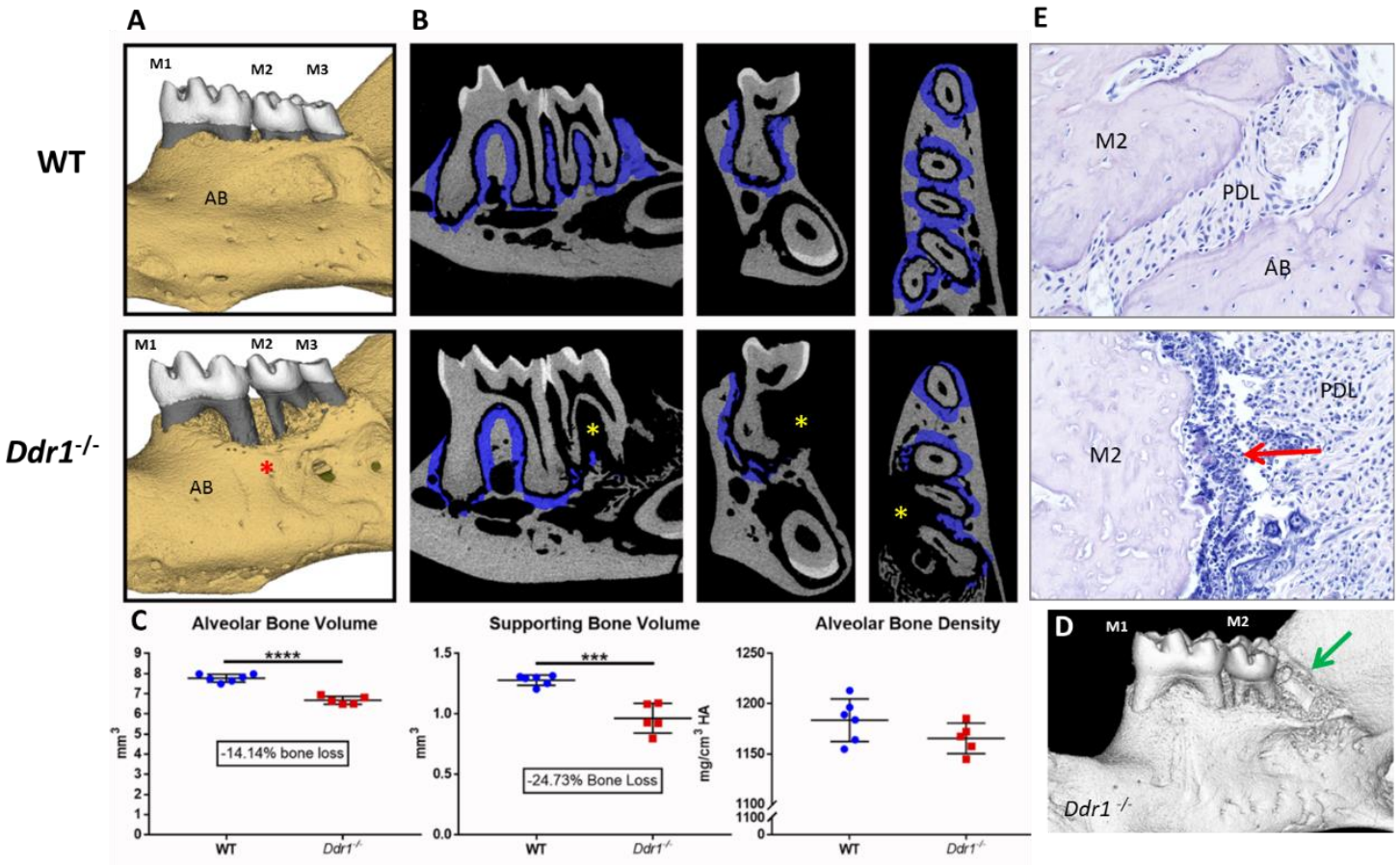

Figure 5. 3D render of molar region and periodontal analysis. (A) 3D render of molar region of WT and *Ddr1*$^{-/-}$ mandibles. (B) The supporting bone (blue) can be seen in three orientations. (C) Overall alveolar bone (AB) volume loss (red/yellow stars in panels A and B) in *Ddr1*$^{-/-}$ mice was 14%, with 24% loss in supporting bone. (D) Loss of supporting bone caused 3rd molar loss (green arrow) in one *Ddr1*$^{-/-}$ mouse. (E) Histology revealed massive infiltration of immune cells (red arrow) into periodontal regions of *Ddr1*$^{-/-}$mandibles in association with

Tooth development in *Ddr1*$^{-/-}$ mice

Histomorphometry and micro-CT were used to analyze tooth development in *Ddr1*$^{-/-}$ mice. Histomorphometric measurements of the mesial root of the 1st molar were made to examine dentin, acellular cementum, PDL, and alveolar bone regions. Length, area, and several other parameters were measured in each region to find any developmental changes in the dimensions of these root and periodontal tissues in *Ddr1*$^{-/-}$ vs. WT mice. Statistical tests confirmed no significant changes in dimensions of any of these tissues ($p > 0.05$ for all) (Table 1) and side-by-side comparison at low (50X) and high (200X) magnification reveals no apparent differences in teeth and supporting structures (Figure 6).

Table 1. Statistical analysis for histomorphometry dimensions of 1st molar.

		WT	KO	P-value
Thickness (μm)	Dentin	162 ± 9	157 ± 18	0.525
	Acellular Cementum	8 ± 3	8 ± 2	0.979
	PDL	82 ± 27	84 ± 20	0.891
	Alveolar Bone	110 ± 20	99 ± 8	0.310
Length (μm)	Tooth - Cellular	1578 ± 144	1585 ± 61	0.922
Area (μm^2)	Cellular Cementum - Buccal	55784 ± 18350	47608 ± 10120	0.420
	Cellular Cementum - Lingual	76105 ± 14073	74658 ± 17888	0.872

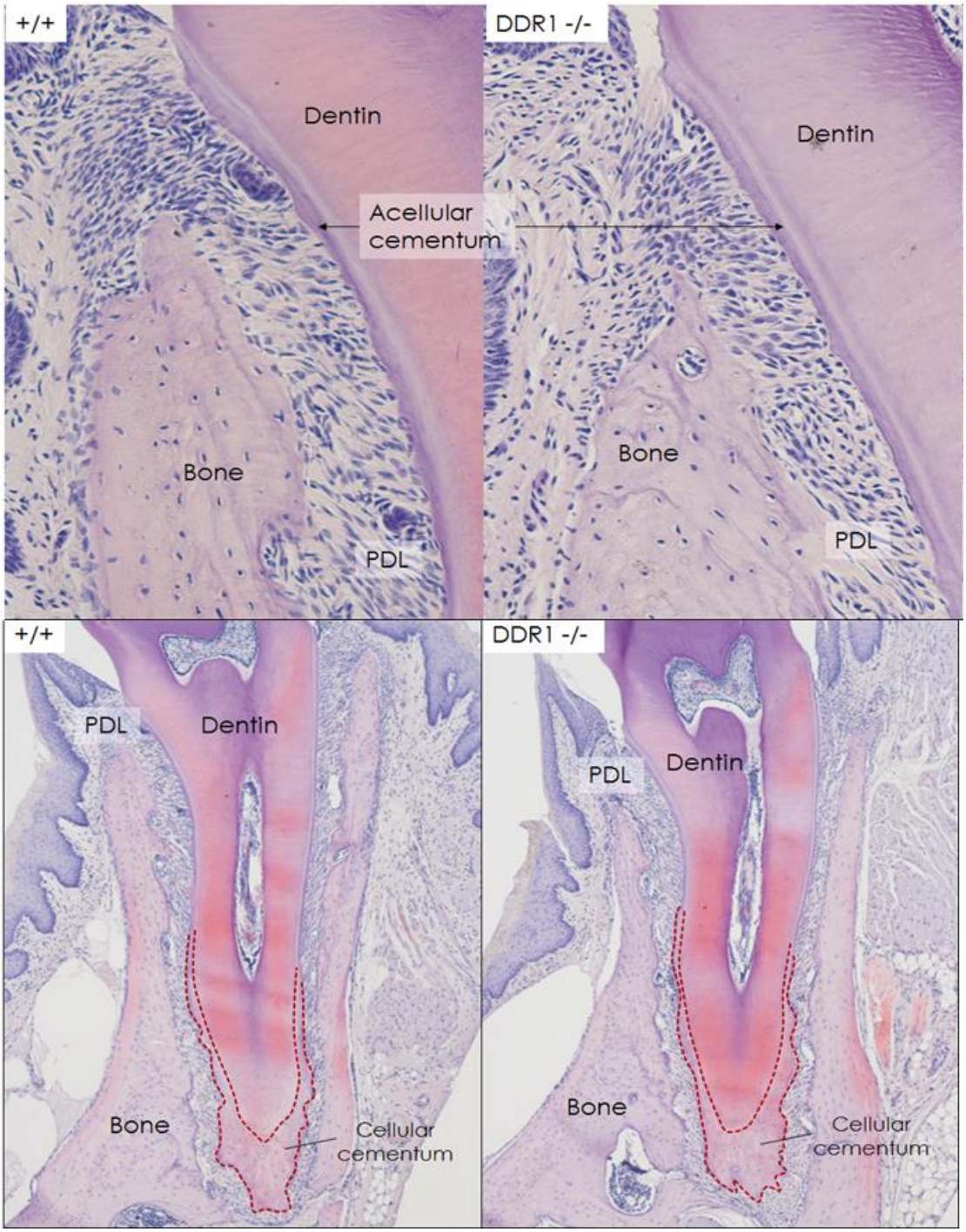

Figure 6. Histology and histomorphometry of molars. Low (50X) and high magnification (200X) H&E stained coronal histological sections of 1st mandibular molars show no apparent changes in tooth anatomy or periodontal attachment *Ddr1*$^{-/-}$ vs. WT mice.

Quantitative micro-CT analysis of volumes and densities of enamel, dentin/cementum, and pulp for 1st, 2nd, and 3rd mandibular molars in WT and *Ddr1*$^{-/-}$ mice were compared (Figure 7). These data showed enamel volume was significantly decreased in 3rd molars ($p < 0.05$), and dentin/cementum volumes were significantly decreased in the 2nd and 3rd molars ($p < 0.01$ and $p < 0.001$, respectively), with no significant changes in pulp volume in *Ddr1*$^{-/-}$ vs. WT mice. No additional volume differences were found and no significant differences were found in the mineral densities of any tissues in *Ddr1*$^{-/-}$ compared to WT mice at 4-6 months.

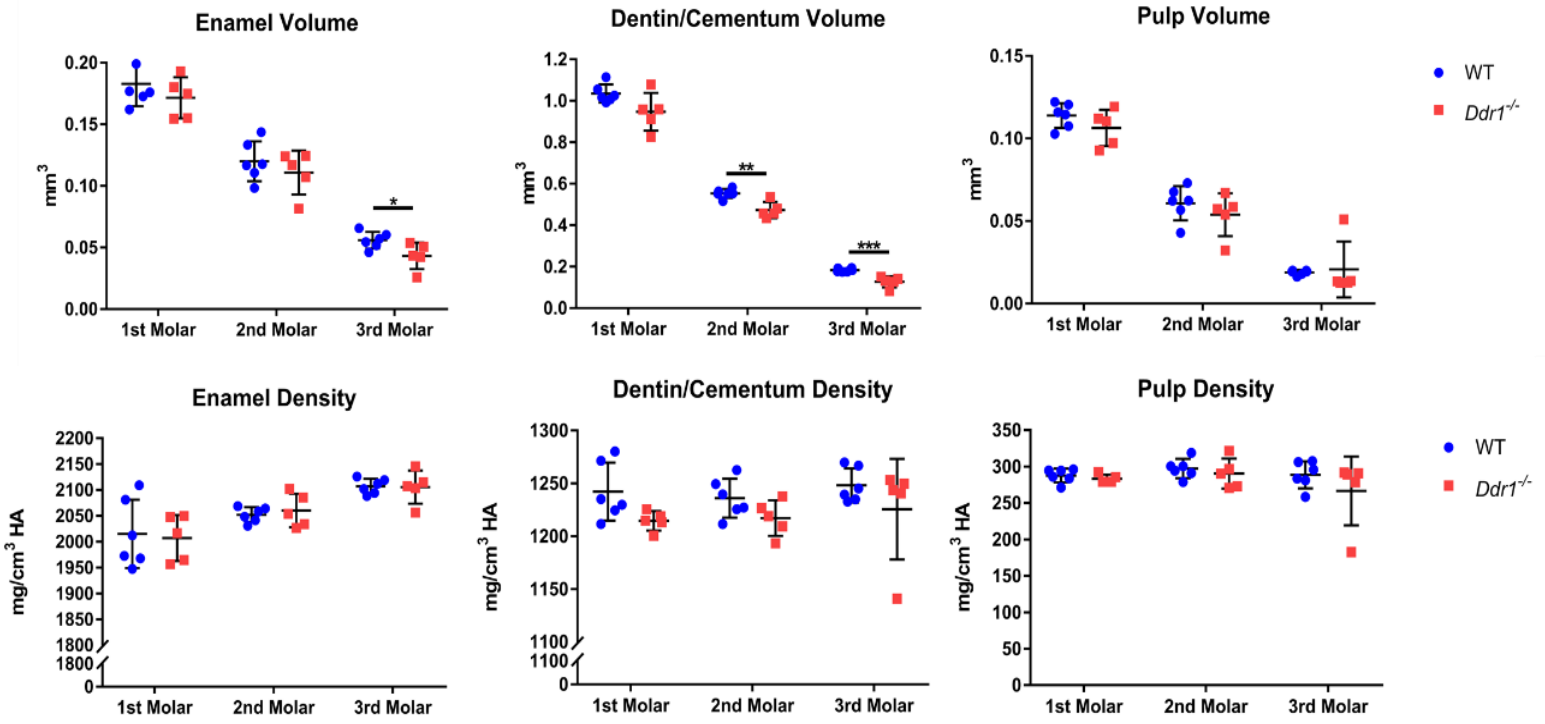

Figure 7. Quantitative micro-computed tomography of molars. Analysis showing volume and density measurements of enamel, dentin/cementum, and pulp for 1st, 2nd, and 3rd molars. Enamel volume was significantly decreased in 3rd molar ($p < 0.05$) and dentin/cementum volumes were significantly decreased in the 2nd and 3rd molars ($p < 0.01$ and $p < 0.001$, respectively).

Chapter 4: Discussion

In this project, we analyzed the spatiotemporal expression of *Ddr1* in dentoalveolar development and described for the first time the effects of genetic ablation of *Ddr1* in development of these oral tissues. We observed *Ddr1* expression strictly in epithelial tissues including the enamel organ and gingiva, but expression was not detected in any of the surrounding ectomesenchymal tissues. *Ddr1*$^{-/-}$ mouse mandibles featured defective condyles and abnormal subchondral bone structure resembling osteoarthritis. *Ddr1*$^{-/-}$ mice featured periodontitis, as indicated by significant alveolar bone loss, inflammatory cell invasion, and tooth loss in severely affected regions. Histomorphometry measurements of oral, dental, and periodontal tissues did not identify significant anatomic differences, including in gingival epithelium. This study suggests important roles for DDR1 in condyle and periodontal function. These insights may potentially inform disease mechanisms, wound healing, novel dental and periodontal therapies, and reconstruction of bone for dental and orthopedic purposes.

Osteoarthritis in Condyle

In 6-month old DDR1 deficient mouse mandibles, the condylar process size was increased, showing signs of osteoarthritis (OA). Our findings of the increase volume of

subchondral and base bone are consistent with other research findings showing other histological signs of OA. Studies with a *Ddr1* deficient mouse have shown incidence of OA in the TMJ as early as 9 weeks of age, displaying collagen type I upregulation, chondrocyte cluster formation, and atypical collagen fibril arrangements (Schminke et al, 2014). This same study also found increased chondrogenesis and expression of DDR2, a key factor in OA. Based on this combined information, we can speculate the mechanism by which OA occurs. The high number of atypical collagen fibrils in the ECM is consistent with what we know of soluble extracellular domains of DDR1 and DDR2 inhibiting collagen fibrillogenesis and enhancing matrix mineralization (Flynn et al, 2011). When DDR1 is deficient, we predict that the collagen fibril network formation will be disrupted and DDR2 will be upregulated to compensate. As a key factor in OA, DDR2 may lead to other histological events happening like the chondrocyte cluster formation. This chondrocyte cluster formation would correlate with our findings of increased subchondral and base bone volumes, but decreased bone to total volume ratio, leading to the enlarged but more porous TMJ region. This mechanism may be studied in more detail by examining ages younger than 4-6 months in order to understand earlier changes in gene expression and condyle architecture associated with onset of OA.

Periodontal Bone Loss

Ddr1 deficient mice showed alveolar bone loss and inflammation in periodontal regions, likely arising from bacteria penetrating the junctional epithelium. The severe onset of periodontitis is a novel finding that suggests DDR1 as being an essential protein for

periodontal function. However, the mechanism(s) by which this occurs remain to be elucidated. In this study, we found *Ddr1* mRNA expression only in the gingival epithelium during development, and not in any of the underlying ectomesenchymal periodontal tissues. This observation suggests loss of DDR1 may compromise epithelial function, possibly allowing bacterial infiltration and subsequent immune response and periodontitis. DDR1 also plays a role in the development of adaptive immune responses, and a previous study reported that DDR1-collagen interactions augment the maturation of dendritic cells through a MAPK (mitogen-activated protein kinase) signaling cascade to contribute to cellular immunity (Matsuyama et al, 2003). This provides an alternative hypothesis that DDR1 may play a role in immune function in the periodontal tissues, and loss of DDR1 somehow reduces efficacy of immune cells to monitor or respond to periodontal pathogens. Signs of periodontitis were noted in the 4-6 month old mice used in our study. In order to further study the underlying mechanisms for periodontal disease, mice at younger ages could be studied to determine structural or cellular defects prior to disease onset, as well as better determining age of onset of periodontitis.

Tooth Development

During tooth development, we report *Ddr1* expression primarily in the enamel organ. Micro-CT analysis revealed normal enamel density in all three molars, and only decreased volume in the 3rd molar of *Ddr1*$^{-/-}$ mice at 4-6 months of age. Despite no developmental expression in pulp and periodontal cells, dentin/cementum volumes of *Ddr1*$^{-/-}$ mice were decreased, but only in 2nd and 3rd molars, though density of

dentin/cementum did not change in *Ddr1*$^{-/-}$ vs. WT mice. Based on epithelial expression of *Ddr1* by ISH, findings in enamel were expected, but those in dentin/pulp were unexpected. *Ddr2* mRNA was present in ectomesenchymal cells, making it a more likely candidate for regulating collagen in development of the tooth root and periodontium. This differential expression of the DDR receptors appears to be context and disease dependent (Camara & Jarai, 2010). Though we do not see obvious developmental expression of *Ddr1* in ectomesenchymal tissues, we cannot rule out a role for DDR1 in their development and homeostasis. It is possible that *Ddr1* deficient mice experience increased collagen production, leading to disordered structure in development of dentin/cementum. Another hypothesis would explain the observed discrepancies in effects on tissues of 2nd and 3rd molars, but not in 1st molars. The periodontitis and immune response appears to localize most severely around the 2nd and 3rd molars, possibly due to local anatomic differences and/or greater probability for food particles to get stuck here and lead to bacterial biofilm formation. Because the mice used in this study were advanced in age (4-6 months), it is possible that onset of periodontitis-like chances at younger ages led to resorption of the hard tissues of 2nd and 3rd molars over time, enough to affect volumes of these tissues. This hypothesis will be explored by examination of mice at earlier ages.

Chapter 5: Conclusions

This research on DDR1 and its involvement within dentoalveolar tissues has increased attentiveness towards the importance of functional collagen reorganization in the oral cavity. We know that collagen is especially important in bones and teeth, and if the structure is disrupted it can lead to various oral degenerative diseases. In this study, we elucidated the role of DDR1 in periodontal function and osteoarthritis of the TMJ as well as its effect during tooth odontogenesis. As a receptor to collagen, DDR1 has been elucidated to play important roles in cell migration, the immune response, and regulators of collagen fibrillization. In future studies, we aim to look at younger mouse models in order to characterize the mechanism of DDR1 in increasing susceptibility to condyle osteoarthritis and periodontitis. We plan to include more mineralization studies to understand whether resorption of enamel and dentin/cementum volumes is occurring. Various histological stains will be performed to investigate the DDR1 role in developing the adaptive immune response towards the periodontitis signs.

It is important to consider what genetic polymorphisms can increase the risk of developing oral degenerative diseases as a means to further understand susceptibility and inform new approaches to regenerating bone and tissue. It also helps to better understand

the ways the body has for restructuring and maintaining structures as well as what mechanisms influence this as the body can have an infinite number of processes. Based on our findings, DDR1 has been found to be important in the function of the condyle of the TMJ and the periodontium. This approach will provide additional mechanistic insights into the importance of DDR1 to development, physiology, and pathology of the dentoalveolar tissues.

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